SEQUENCE LISTING

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<110> Tang, Jordan J.N.
      Hong, Lin
      Ghosh, Arun K.
<120> Inhibitors of Memapsin 2 and Use Thereof
<130> OMRF 182
<140> Not Yet Assigned
<141> 2000-06-27
<150> 60/141,363
<151> 1999-06-28
<150> 60/168,060
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<150> 60/177,836
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 cccggccgga ggggcagctt tgtggagatg gtggacaacc tgaggggcaa gtcggggcag 180
 ggctactacg tggagatgac cgtgggcagc ccccgcaga cgctcaacat cctggtggat 240
 acaggcagca gtaactttgc agtgggtgct gcccccacc ccttcctgca tcgctactac 300
 cagaggeage tgtccageac atacegggae etceggaagg gtgtgtatgt geeetacace 360
 cagggcaagt gggaagggga getgggcace gaeetggtaa geateeecca tggeeecaac 420
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gtcactgtgc gtgccaacat tgctgccatc actgaatcag acaagttctt catcaacggc 480 tccaactggg aaggcatcct ggggctggcc tatgctgaga ttgccaggcc tgacgactcc 540 ctggagcctt tctttgactc tctggtaaag cagacccacg ttcccaacct cttctccctg 600

cagetttgtg gtgctggctt ccccctcaac cagtctgaag tgctggcctc tgtcggaggg 660 agcatgatca ttggaggtat cgaccactcg ctgtacacag gcagtctctg gtatacaccc 720 atccggcggg agtggtatta tgaggtgatc attgtgcggg tggagatcaa tggacaggat 780 ctgaaaatgg actgcaagga gtacaactat gacaagagca ttgtggacag tggcaccacc 840 aaccttcgtt tgcccaagaa agtgtttgaa gctgcagtca aatccatcaa ggcagcctcc 900 tccacggaga agttccctga tggtttctgg ctaggagagc agctggtgtg ctggcaagca 960 ggcaccaccc cttggaacat tttcccagtc atctcactct acctaatggg tgaggttacc 1020 aaccagteet teegeateae cateetteeg cageaatace tgeggecagt ggaagatgtg 1080 gccacgtccc aagacgactg ttacaagttt gccatctcac agtcatccac gggcactgtt 1140 atgggagctg ttatcatgga gggcttctac gttgtctttg atcgggcccg aaaacgaatt 1200 ggctttgctg tcagcgcttg ccatgtgcac gatgagttca ggacggcagc ggtggaaggc 1260 ccttttgtca ccttggacat ggaagactgt ggctacaaca ttccacagac agatgagtca 1320 acceteatga ceatageeta tgteatgget gecatetgeg ceetetteat getgeeacte 1380 tgcctcatgg tgtgtcagtg gcgctgcctc cgctgcctgc gccagcagca tgatgacttt 1440 gctgatgaca tctccctgct gaagtgagga ggcccatggg cagaagatag agattcccct 1500 ggaccacacc tccgtggttc actttggtca caagtaggag acacagatgg cacctgtggc 1560 cagagcacct caggaccctc cccacccacc aaatgcctct gccttgatgg agaaggaaaa 1620 ggctggcaag gtgggttcca gggactgtac ctgtaggaaa cagaaaagag aagaaagaag 1680 cactetgetg gegggaatae tettggteae etcaaattta agtegggaaa ttetgetget 1740 tgaaacttca gccctgaacc tttgtccacc attcctttaa attctccaac ccaaagtatt 1800 cttctttct tagtttcaga agtactggca tcacacgcag gttaccttgg cgtgtgtccc 1860 tgtggtaccc tggcagagaa gagaccaagc ttgtttccct gctggccaaa gtcagtagga 1920 gaggatgcac agtttgctat ttgctttaga gacagggact gtataaacaa gcctaacatt 1980 ggtgcaaaga ttgcctcttg aattaaaaaa aaactagatt gactatttat acaaatgggg 2040 gcggctggaa agaggagaag gagagggagt acaaagacag ggaatagtgg gatcaaagct 2100 aggaaaggca gaaacacaac cactcaccag tcctagtttt agacctcatc tccaagatag 2160 catcccatct cagaagatgg gtgttgtttt caatgttttc ttttctgtgg ttgcagcctg 2220 accaaaagtg agatgggaag ggcttatcta gccaaagagc tcttttttag ctctcttaaa 2280 tgaagtgccc actaagaagt tccacttaac acatgaattt ctgccatatt aatttcattg 2340 tetetatetg aaccaecett tattetaeat atgataggea geaetgaaat ateetaaece 2400 cctaagctcc aggtgccctg tgggagagca actggactat agcagggctg ggctctgtct 2460 tectggteat aggeteacte tittececeaa atetteetet ggagetitige ageeaaggtg 2520 ctaaaaggaa taggtaggag acctcttcta tctaatcctt aaaagcataa tgttgaacat 2580 tcattcaaca gctgatgccc tataacccct gcctggattt cttcctatta ggctataaga 2640 agtagcaaga tetttacata atteagagtg gttteattge etteetacee tetetaatgg 2700 cccctccatt tatttgacta aagcatcrca cagtggcact agcattatac caagagtatg 2760 agaaatacag tgctttatgg ctctaacatt actgccttca gtatcaaggc tgcctggaga 2820 aaggatggca gcctcagggc ttccttatgt cctccaccac aagagctcct tgatgaaggt 2880 catctttttc ccctatcctg ttcttcccct ccccgctcct aatggtacgt gggtacccag 2940 gctggttctt gggctaggta gtggggacca agttcattac ctccctatca gttctagcat 3000 agtaaactac ggtaccagtg ttagtgggaa gagctgggtt ttcctagtat acccactgca 3060 tcctactcct acctggtcaa cccgctgctt ccaggtatgg gacctgctaa gtgtggaatt 3120 acctgataag ggagagggaa atacaaggag ggcctctggt gttcctggcc tcagccagct 3180 gcccmcaagc cataaaccaa taaamcaaga atactgagtc taaaaaaaaa aaaaaaaaa 3240 3252 aaaaaaaaa aa

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<213> Homo sapiens

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<223> Purified Memapsin 2

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<223> Amino Acids 28-48 are remnant putative propeptide residues

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<223> Amino Acids 58-61, 78, 80, 82-83, 116, 118-121, 156, 166, 174, 246, 274, 276, 278-281, 283, and 376-377 are residues in contact with the OM99-2 inhibitor

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<223> Amino acids 54-57, 61-68, 73-80, 86-89, 109-111, 113-118, 123-134, 143-154, 165-168, 198-202, and 220-224 are N-lobe Beta Strands

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<223> Amino Acids 184-191 and 210-217 are N-lobe Helices

<220>

<220>

<223> Amino Acids 286-299, 307-310, 350-353, 384-387, and 427-431 are C-lobe Helices

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Leu Arg Ser Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg
20 25 30

Glu Thr Asp Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val
35 40 45

Glu Met Val Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val
50 55 60

Glu Met Thr Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp 65 70 75 80

Thr Gly Ser Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu

His Arg Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg
100 105 110

Lys Gly Val Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu 115 120 125

Gly Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg 130 135 140

Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly
145 150 155 160

Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg 165 170 175

Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr
180 185 190

His Val Pro Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro 195 200 205

Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile 210 215 220

Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro 225 230 235 240

Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile
245 250 255

Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys 260 265 270

Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val 275 280 285

Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys 290 295 300

Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala 305 310 315 320 Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met 325 330 335

Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln 340 345 350

Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr 355 360 365

Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val 370 375 380

Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile 385 390 395 400

Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala 405 410 415

Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr 420 425 430

Asn Ile Pro Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val 435 440 445

Met Ala Ala Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val 450 455 460

Cys Gln Trp Arg Cys Leu Arg Cys Leu Arg Gln Gln His Asp Asp Phe 465 470 475 480

Ala Asp Asp Ile Ser Leu Leu Lys
485

<210>...3

<211> 503

<212> PRT

<213> Homo sapiens

<220>

<223> Pro-memapsin 2

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<223> Amino Acids 1-15 are vector-derived residues

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<223> Amino Acids 16-64 are a putative pro peptide

<220>

<223> Amino Acids 1-13 are the T7 promoter

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<223> Amino Acids 16-456 are Pro-memapsin 2-T1

<220>

<223> Amino Acids 16-421 are Promemapsin 2-T2

<400> 3

Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Gly Ser Met Ala

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Gly Val Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu 20 25 30

Arg Ser Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu 35 40 45

Thr Asp Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu
50 55 60

Met Val Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu 65 70 75 80

Met Thr Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr 85 90 95

Gly Ser Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His
100 105 110

Arg Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys
115 120 125

Gly Val Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly
130 135 140

Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala
145 150 155 160

Asn Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser 165 170 175

Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro 180 185 190

Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His
195 200 205

Val Pro Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu 210 215 220

Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly 225 230 235 240

Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile
245 250 255

Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn 260 265 270

Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser 275 280 285

Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe 290 295 300

Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe 305 310 315 320

Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly
325 330 335

Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly 340 345 350

Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr 355 360 365

Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys 370 375 380

Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile 385 390 395 400

Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly 405 410 415

Phe Ala Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala 420 425 430

Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn 435 440 445

Ile Pro Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met 450 455 460

Ala Ala Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val Cys 475 480 465 470 Gln Trp Arg Cys Leu Arg Cys Leu Arg Gln Gln His Asp Asp Phe Ala 495 485 490 Asp Asp Ile Ser Leu Leu Lys 500 <210> 4 <211> 10 <212> PRT <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Primer <400> 4 Ser Glu Val Lys Met Asp Ala Glu Phe Arg 5 <210> 5 <211> 10 <212> PRT <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic Peptide <400> 5 Ser Glu Val Asn Leu Asp Ala Glu Phe Arg 1 5 <210> 6 <211> 8 <212> PRT

<400> 6

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Peptide

<223> Description of Artificial Sequence: Synthetic

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<211> 12
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Leu Val Asn Met Ala Glu Gly Asp
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<210> 13	
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(22) Deportation of Withingt Bedneuce: Little	
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<210> 19	
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Caron Bollon Co. Managara Bollon Co.	
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catatggcgg gagtgctgcc tgcccac	27
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ggaboccae bocagoaggg agabgocabo agoaaagb	
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Insulin B-chain	
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His Leu Xaa Gly Ser His Leu Val
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Xaa Gly Glu Arg Gly Phe Phe Tyr
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       79-87, 89-91, 99-106, 119-122, 150-154, 164-167,
       180-183, 191-194, 196-199, 201-204, 210-214,
       221-223, 258-262, 265-269, and 275-278 are Beta
       Strands
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 <223> Amino acids 281-284, 286-288, 298-301, 310-315,
        and 319-324 are Beta strands
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  <223> Amino acids 48-51, 111-114, 136-142, 225-234,
        249-254, 271-274, and 303-306 are Helices
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  <223> Amino acids 12-13, 30, 32, 34-35, 73-77, 111, 117,
        120, 189, 213, 215, 217-220, 287, 289, 291, 298,
        and 300 are residues in contact with pepstatin.
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<220> <223> Pepsin

<400> 31

Val Asp Glu Gln Pro Leu Glu Asn Tyr Leu Asp Met Glu Tyr Phe Gly
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Thr Ile Gly Ile Gly Thr Pro Ala Gln Asp Phe Thr Val Val Phe Asp 20 25 30

Thr Gly Ser Ser Asn Leu Trp Val Pro Ser Val Tyr Cys Ser Ser Leu 35 40 45

Ala Cys Thr Asn His Asn Arg Phe Asn Pro Glu Asp Ser Ser Thr Tyr
50 55 60

Gln Ser Thr Ser Glu Thr Val Ser Ile Thr Tyr Gly Thr Gly Ser Met
65 70 75 80

Thr Gly Ile Leu Gly Tyr Asp Thr Val Gln Val Gly Gly Ile Ser Asp 85 90 95

Thr Asn Gln Ile Phe Gly Leu Ser Glu Thr Glu Pro Gly Ser Phe Leu
100 105 110

Tyr Tyr Ala Pro Phe Asp Gly Ile Leu Gly Leu Ala Tyr Pro Ser Ile 115 120 125

Ser Ser Ser Gly Ala Thr Pro Val Phe Asp Asn Ile Trp Asn Gln Gly
130 135 140

Leu Val Ser Gln Asp Leu Phe Ser Val Tyr Leu Ser Ala Asp Asp Gln 145 150 155 160

Ser Gly Ser Val Val Ile Phe Gly Gly Ile Asp Ser Ser Tyr Tyr Thr 165 170 175

Gly Ser Leu Asn Trp Val Pro Val Thr Val Glu Gly Tyr Trp Gln Ile 180 185 190

Thr Val Asp Ser Ile Thr Met Asn Gly Glu Ala Ile Ala Cys Ala Glu 195 200 205

Gly Cys Gln Ala Ile Val Asp Thr Gly Thr Ser Leu Leu Thr Gly Pro 210 215 220

Thr Ser Pro Ile Ala Asn Ile Gln Ser Asp Ile Gly Ala Ser Glu Asn

225 230 235 240

Ser Asp Gly Asp Met Val Val Ser Cys Ser Ala Ile Ser Ser Leu Pro 245 250 255

Asp Ile Val Phe Thr Ile Asn Gly Val Gln Tyr Pro Val Pro Pro Ser 260 265 270

Ala Tyr Ile Leu Gln Ser Glu Gly Ser Cys Ile Ser Gly Phe Gln Gly
275 280 285

Met Asn Leu Pro Thr Glu Ser Gly Glu Leu Trp Ile Leu Gly Asp Val 290 295 300

Phe Ile Arg Gln Tyr Phe Thr Val Phe Asp Arg Ala Asn Asn Gln Val 305 310 315 320

Gly Leu Ala Pro Val Ala 325